



AMENDMENTS TO THE CLAIMS

1. **(Currently amended)** A base type determination method for determining a base type of a monobasic substituted region of a target nucleic acid, the method comprising the steps of:

(a) preparing a solution containing a target double-stranded nucleic acid having the monobasic substituted region, a base type determination primer, a DNA polymerase, and dNTPs;

(b) hybridizing the base type determination primer to the target double-stranded nucleic acid in the solution, and causing a primer extension reaction to start progressing from the base type determination primer; and

(c) analyzing the degree of progress of the primer extension reaction to determine the base type of the substituted region,

wherein the base type determination primer consists of a first single-stranded nucleic acid which is capable of, when hybridizing to the target double-stranded nucleic acid, hybridizing to one of two strands of the target double-stranded nucleic acid such that a 3' terminal of the primer corresponds to the substituted region of said one strand of the target double-stranded nucleic acid, and

wherein the first single-stranded nucleic acid consists of:

a substitution corresponding region which is located at the 3' terminal of the first single-stranded nucleic acid and consists of one base complementary to any one of predictable types of bases in the substituted region of said one strand of the target double-stranded nucleic acid;

an uncomplementary region which is located adjacent to the substitution corresponding region on a 5' terminal side thereof and consists of two bases, which are located in the second and third positions, respectively, from the 3' terminal of the first single-stranded nucleic acid, and is said two bases are uncomplementary, respectively, to said one strand of the target double-stranded nucleic acid; and

a complementary region which is located adjacent to the uncomplementary region on the 5' terminal side thereof and consists of five or more bases complementary to said one strand of the target double-stranded nucleic acid.

2. (Original) The base type determination method according to claim 1, wherein the DNA polymerase has substantially no 3' → 5' exonuclease activity.

3. (Original) The base type determination method according to claim 1, wherein the first single-stranded nucleic acid is a DNA.

4. (Original) The base type determination method according to claim 1, wherein in the step (a), the solution further contains a reverse primer consisting of a single-stranded nucleic acid capable of hybridizing to the other strand of the target double-stranded nucleic acid, and

wherein in the step (b), the primer extension reaction is caused to progress using a base sequence amplifying method selected from the group consisting of a PCR, an SDA, an RCR, an LAMP, and a TMA.

5. (Original) The base type determination method according to claim 4, wherein in the step (c), the base type of the substituted region is determined based on a difference in progress of the primer extension reaction.

6. (Original) The base type determination method according to claim 4, wherein in the step (c), the degree of progress of the primer extension reaction is analyzed by using a method selected from the group consisting of electrophoresis, mass analysis, and liquid chromatography to measure the amount of amplification of a base sequence amplified by the base sequence amplifying method.

7. (Original) The base type determination method according to claim 1, wherein in the step (c), the degree of progress of the primer extension reaction is analyzed by measuring the amount of pyrophosphoric acid generated by the primer extension reaction.

8. (Original) The base type determination method according to claim 4, wherein in the step (c), the amount of amplification of a base sequence amplified by the base sequence amplifying method is measured by measuring the amount of pyrophosphoric acid generated by the primer extension reaction.

9. (Original) The base type determination method according to claim 4, wherein in the step (c), the amount of amplification of a base sequence amplified by the base sequence amplifying method is quantitatively analyzed to determine the base type of the substituted region.

10. (Original) The base type determination method according to claim 7,
wherein measurement of the amount of pyrophosphoric acid includes the steps of:
 converting the pyrophosphoric acid into an inorganic phosphoric acid within a sample containing at least a portion of the solution resulted from the step (b);
 providing the sample to a measurement system including glyceraldehyde 3-phosphate, oxidized nicotinamide adenine dinucleotide, glyceraldehyde 3-phosphatedehydrogenase, and at least one electron-transfer mediator; and
 measuring a value of current generated in the measurement system, and
wherein the value of current indicates a concentration of the pyrophosphoric acid in the sample.

11. (Original) The base type determination method according to claim 10, wherein said at least one electron-transfer mediator is selected from the group consisting of ferricyanide, 1,2-naphthoquinone-4-sulfonic acid, 2,6-dichlorophenol- indophenol, dimethylbenzoquinone, 1-methoxy-5- methylphenazinium sulfate, methylene blue, gallocyanine, thionine, phenazine methosulfate, and meldora blue.

12. (Previously presented) The base type determination method according to claim 10, wherein the measurement system further includes diaphorase.

13. (Original) The base type determination method according to claim 10, wherein the pyrophosphoric acid is converted into the inorganic phosphoric acid by causing the pyrophosphoric acid to react with pyrophosphatase in the sample.

14. (Original) The base type determination method according to claim 7,
wherein measurement of the amount of the pyrophosphoric acid includes the steps of:
 placing a sample including at least a portion of a solution resulted from the step
(b) in one region of a measurement system having at least two regions divided by a membrane
which holds H^+ -pyrophosphatase and has a limited permeability to H^+ ; and
 measuring a change in concentration of H^+ in either one of said at least two
regions of the measurement system, and
 wherein the degree of the change in concentration of H^+ indicates the concentration of the
pyrophosphoric acid in the sample.

15. (Original) The base type determination method according to claim 14,
wherein the measurement of the pyrophosphoric acid includes the steps of:
 providing the sample including at least a portion of a solution resulted from the
step (b) to a measurement system including an artificial or natural membrane vesicle containing
 H^+ -pyrophosphatase therein; and
 measuring the change in concentration of H^+ in the inside or outside of the
membrane vesicle, and
 wherein the degree of the change in concentration of H^+ indicates the concentration of the
pyrophosphoric acid in the sample.

16. (Original) The base type determination method according to claim 14, wherein the
change in concentration of H^+ is measured by either a method which measures an optical change
converted from the change in concentration of H^+ or a method which measures an electrical
change converted from the change in concentration of H^+ .

17. (Original) The base type determination method according to claim 16, wherein the method which measures an optical change uses a pH test paper, a pH-sensitive dye, or a membrane potential-sensitive dye.

18. (Original) The base type determination method according to claim 16, wherein the method which measures an electrical change is selected from the group consisting of a metal electrode method, a glass electrode method, an ISFET electrode method, a patch-clamp method, and an LAPS method.

19. (Original) The base type determination method according to claim 17, wherein the method which measures an optical change uses the pH-sensitive dye to measure the change in concentration of H^+ in the inside of the membrane vesicle.

20. (Currently amended) The base type determination method according to claim 1, wherein in the step (a), the solution further contains a second base type determination primer, wherein the second base type determination primer consists of a second single-stranded nucleic acid which is capable of, when hybridizing to the target double-stranded nucleic acid, hybridizing to one of two strands of the target double-stranded nucleic acid which is the same strand as that to which the first base type determination primer is supposed to hybridize, such that a 3' terminal of the second base type determination primer corresponds to the substituted region of said one strand, and

wherein the second single-stranded nucleic acid includes:

a second substitution corresponding region located at the 3' terminal of the second single-stranded nucleic acid and consisting of one base which is complementary to any one of predictable types of bases in the substituted region of the target double-stranded nucleic acid and is different in type from said one base of the substitution corresponding region of the first single-stranded nucleic acid;

a second uncomplementary region which is adjacent to the second substitution corresponding region on the 5' terminal side and consists of two bases, which are located in the second and third positions, respectively, from the 3' terminal of the second single-stranded nucleic acid, ~~and is~~ said two bases are uncomplementary to said one strand of the target double-stranded nucleic acid; and

a second complementary region which is adjacent to the second uncomplementary region on the 5' terminal side and consists of five or more bases complementary to said one strand of the target double-stranded nucleic acid.

21. (Original) The base type determination method according to claim 20, wherein the first single-stranded nucleic acid and the second single-stranded nucleic acid are different in length from each other.

22. (Original) The base type determination method according to claim 20, wherein the first single-stranded nucleic acid and the second single-stranded nucleic acid are labeled by their respective fluorescences which are different in wavelength.

23. (Withdrawn) A base type determination primer for determining a base type of a monobasic substituted region of a target nucleic acid,

wherein the primer consists of a single-stranded nucleic acid which is capable of hybridizing to the target nucleic acid such that a 3' terminal of the primer corresponds to the substituted region of the target nucleic acid, and

wherein the single-stranded nucleic acid includes:

a substitution corresponding region which is located at the 3' terminal and consists of one base complementary to any one of predictable types of bases in the substituted region of the target nucleic acid;

an uncomplementary region which is located adjacent to the substitution corresponding region on a 5' terminal side thereof and consists of two bases uncomplementary to the target nucleic acid; and

a complementary region which is located adjacent to the uncomplementary region on the 5' terminal side thereof and consists of five or more bases complementary to the target nucleic acid.

24. (Withdrawn) A base type determination reagent kit for determining a base type of a monobasic substituted region of a target nucleic acid, the kit comprising a base type determination primer, a DNA polymerase, and dNTPs,

wherein the primer consists of a first single-stranded nucleic acid which is capable of hybridizing to the target nucleic acid such that a 3' terminal of the primer corresponds to the substituted region of the target nucleic acid, and

wherein the first single-stranded nucleic acid includes:

a substitution corresponding region which is located at the 3' terminal and consists of one base complementary to any one of predictable types of bases in the substituted region of the target nucleic acid;

an uncomplementary region which is located adjacent to the substitution corresponding region on a 5' terminal side thereof and consists of two bases uncomplementary to the target nucleic acid; and

a complementary region which is located adjacent to the uncomplementary region on the 5' terminal side thereof and consists of five or more bases complementary to the target nucleic acid.

25. (Withdrawn) The base type determination reagent kit according to claim 24, wherein the DNA polymerase has substantially no 3' → 5' exonuclease activity.

26. (Withdrawn) The base type determination reagent kit according to claim 24, wherein the first single-stranded nucleic acid is a DNA.

27. (Withdrawn) The base type determination reagent kit according to claim 24, further comprising a reverse primer.

28. (Withdrawn) The base type determination reagent kit according to claim 24, further comprising pyrophosphatase.

29. (Withdrawn) The base type determination reagent kit according to claim 28, further comprising glyceraldehyde 3-phosphate, oxidized nicotinamide adenine dinucleotide, glyceraldehyde 3-phosphatedehydrogenase, and at least one electron-transfer mediator.

30. (Withdrawn) The base type determination reagent kit according to claim 29, further comprising diaphorase.

31. (Withdrawn) The base type determination reagent kit according to claim 29, wherein said at least one electron-transfer mediator is selected from the group consisting of ferricyanide, 1,2-naphthoquinone-4-sulfonic acid, 2,6-dichlorophenol- indophenol, dimethylbenzoquinone, 1-methoxy-5- methylphenazinium sulfate, methylene blue, gallocyanine, thionine, phenazine methosulfate, and meldora blue.

32. (Withdrawn) The base type determination reagent kit according to claim 24, further comprising H⁺-pyrophosphatase.

33. (Withdrawn) The base type determination reagent kit according to claim 32, further comprising a pH test paper, a pH-sensitive dye, or a membrane potential-sensitive dye.

34. (Withdrawn) The base type determination reagent kit according to claim 24, further comprising a second base type determination primer,

wherein the second base type determination primer consists of a second single-stranded nucleic acid capable of hybridizing to the target nucleic acid such that the 3' terminal corresponds to the substituted region of the same strand as that to which the first base type determination primer is supposed to hybridize, and

wherein the second single-stranded nucleic acid includes:

a second substitution corresponding region located at the 3' terminal and consisting of one base which is complementary to any one of predictable types of bases in the substituted region of the target nucleic acid and is different in type from said one base of the substitution corresponding region of the first single-stranded nucleic acid;

a second uncomplementary region which is located adjacent to the second substitution corresponding region on a 5' terminal side thereof and consists of two bases uncomplementary to the target nucleic acid; and

a second complementary region which is located adjacent to the second uncomplementary region on the 5' terminal side thereof and consists of five or more bases complementary to the target nucleic acid.

35. (Withdrawn) The base type determination reagent kit according to claim 34, wherein the first single-stranded nucleic acid and the second single-stranded nucleic acid are different in length from each other.

36. (Withdrawn) The base type determination reagent kit according to claim 34, wherein the first single-stranded nucleic acid and the second single-stranded nucleic acid are labeled by their respective fluorescences which are different in wavelength.